

**U.S. DEPARTMENT OF COMMERCE
National Technical Information Service**

PB-294 796

The Effects of Reactions of Chlorine Dioxide in Water. Volume 2

SRI International, Menlo Park, CA

Prepared for

Environmental Protection Agency, Washington, DC Office of Drinking Water

Mar 79

TECHNICAL REPORT DATA
(Please read instructions on the reverse before completing)

1. REPORT NO.		2.		3. RECEIPT NO. (If applicable)	
4. TITLE AND SUBTITLE				5. REPORT DATE	
The Effects of Reactions of Chlorine Dioxide in Water. Volume 2				March 1979	
7. AUTHOR(S)				6. PERFORMING ORGANIZATION CODE	
V.F. Simmon, R.J. Spangord, S.L. Eckford and V. McClurg				LSU-4316	
9. PERFORMING ORGANIZATION NAME AND ADDRESS				8. PERFORMING ORGANIZATION REPORT NO.	
SRI International 333 Ravenswood Ave. Menlo Park, CA 94025					
10. PROGRAM ELEMENT NO.				11. CONTRACT/GRANT NO.	
				68-01-2894	
12. SPONSORING AGENCY NAME AND ADDRESS				13. TYPE OF REPORT AND PERIOD COVERED	
Office of Drinking Water EPA Washington, D.C. 20460				Final	
				14. SPONSORING AGENCY CODE	
15. SUPPLEMENTARY NOTES					
16. ABSTRACT					
This study reports the results of the evaluation of twenty-six compounds for mutagenic activity before and after treatment with chlorine dioxide. Two biological assays, the Ames <u>Salmonella</u>/microsome and the yeast <u>Saccharomyces cerevisiae</u> D3 assays, were used to determine the mutagenic activity of each compound.					
17. KEY WORDS AND DOCUMENT ANALYSIS					
a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS		c. COSATI Field/Group	
18. DISTRIBUTION STATEMENT		19. SECURITY CLASS (This Report)		21. NO. OF PAGES	
Release unlimited				183	
		20. SECURITY CLASS (This page)		22. PRICE	
				A09-A01	

PB 294796

THE EFFECTS OF REACTIONS OF CHLORINE DIOXIDE IN WATER

Volume 2

Final Report

March 1979

By: Vincent F. Simmon, Ronald J. Spanggord,
Sharon L. Eckford, and Vernon McClurg

Prepared for:

Environmental Protection Agency
Waterside Mall
401 M Street, SW
Washington, D.C. 20406

Attention:

Dr. Joseph Cotruvo-WH550
Room E1111
Office of Drinking Water

Environmental Protection Agency
Library Systems Branch, Room 2404
401 M Street, SW, WSM PM-213
Washington, D.C. 20460

SRI International
333 Ravenswood Avenue
Menlo Park, California 94025
(415) 326-6200
Cable: SRI INTL MNP
TWX: 910-373-1246

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161





THE EFFECTS OF REACTIONS OF CHLORINE DIOXIDE IN WATER

Volume 2

Final Report

March 1979

**By: Vincent F. Simmon, Ronald J. Spanggord,
Sharon L. Eckford, and Vernon McClurg**

Prepared for:

**Environmental Protection Agency
Waterside Mall
401 M Street, SW
Washington, D.C. 20406**

Attention:

**Dr. Joseph Cotruvo-WH550
Room E1111
Office of Drinking Water**

Contract No. 68-01-2894

SRI Project LSU-4316

Approved by:

**David C. L. Jones, Director
Toxicology Laboratory**

**W. A. Skinner, Executive Director
Life Sciences Division**

ia

CONTENTS

INTRODUCTION	1
METHODS	3
Chemistry	3
Microbiology	5
RESULTS	11
Chemical Results	11
Microbiological Results	16
DISCUSSION	21
EXECUTIVE SUMMARY	23
REFERENCES	25
APPENDIX: Comparative Study of Three Oxidizing Agents	A-1

INTRODUCTION

Water purification has been commonly achieved through chlorination techniques. However, low levels of halogenated hydrocarbons have been found in drinking water, which may be due to the use of chlorine during the process of water purification. Several of these halogenated hydrocarbons are known to be mutagenic and/or carcinogenic.^{1, 2}

Chlorine dioxide may be a possible alternative for water purification, thus avoiding the generating of chloro-organics. In this study, we evaluated twenty-six compounds for mutagenic activity before and after treatment with chlorine dioxide. The chemicals tested were ethanol, acetic acid, nitrilotriacetic acid trisodium salt monohydrate, phenol, diethylamine, hydroquinone, caffeine, benzidine•2HCl, urea, benzene, oleic acid, humic acid, α -D-glucose, cholesterol, thymine, benzo(a)pyrene, cysteine, phenylalanine, aldrin, 1,1-diphenylhydrazine, glycylglycine, dibutylphthalate, citral, coprosterol, glycerol, and benzoic acid.

Two biological assays were used to determine the mutagenic activity of each compound. One was the Ames Salmonella/microsome assay for induction of reverse mutation in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. The other was the yeast Saccharomyces cerevisiae D3 assay for mitotic recombination. Both assays were performed in the presence and in the absence of a rat liver homogenate metabolic activation system.

Chemical analyses were performed to confirm oxidation of the starting materials and to identify transformation products.

This study is designed to parallel the ozonation study (draft report entitled "I. The Effects of Ozonation Reactions in Water, Volume 1," submitted to EPA in February 1977). It assesses the biological hazard of the use of chlorine dioxide as an alternative disinfection

technique in the purification of water. It also employs the use of rapid microbial mutagenicity assays as an estimate of potential biological hazard.

At the request of EPA, a comparative study of three oxidants--chlorine, ozone, and chlorine dioxide--was performed. The chemical degradation products and biological responses for three selected organic compounds--humic acid, phenol, and benzidine--were compared before and after treatment with the three oxidizing agents. The details and results are given in the Appendix.

METHODS

Chemistry

The objectives of the chemical portion of this study were (a) to provide solutions of selected organic compounds for mutagenic screening under pre- and post-chlorine dioxide conditions and (b) to chemically identify transformation products in solutions found to be biologically active.

Preparation of Chlorine Dioxide

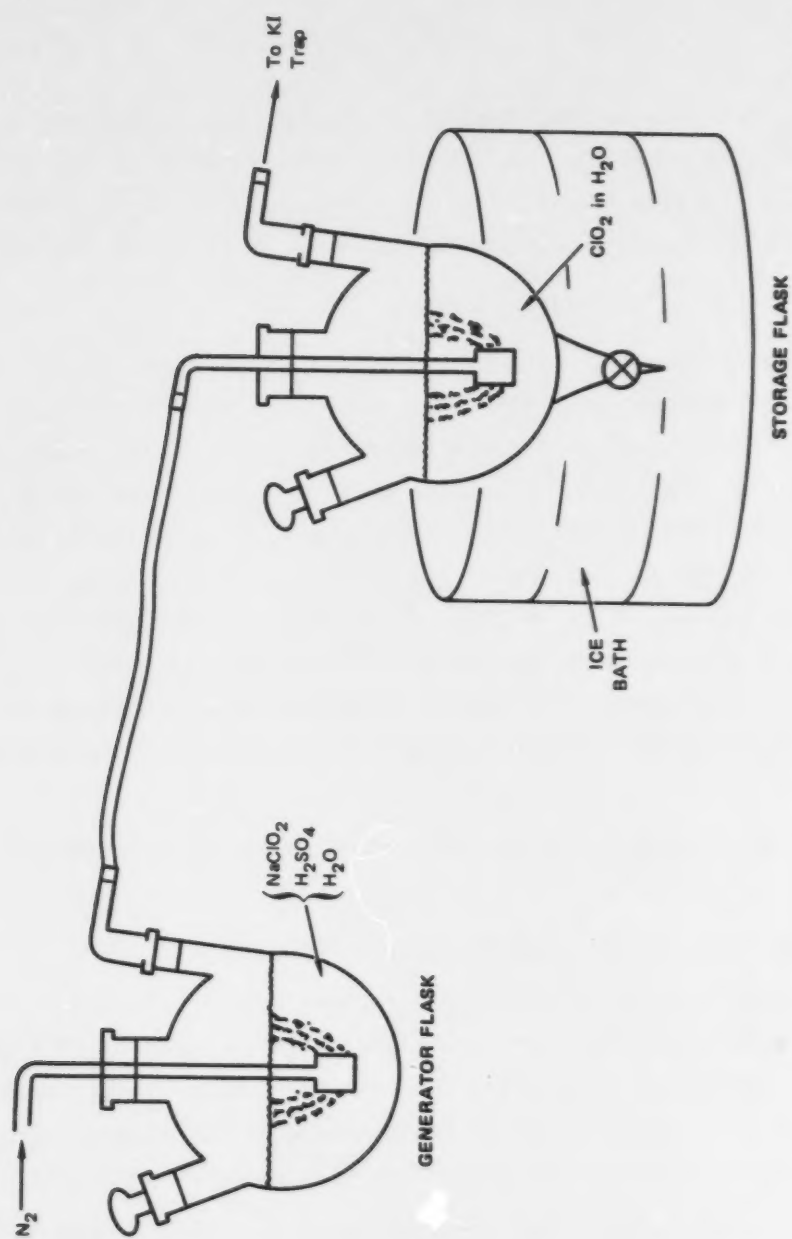
The generation of chlorine dioxide (ClO_2) was carried out in a 250-ml, 3-neck, round-bottom pyrex flask equipped with a fritted glass inlet tube (Fig. 1). The flask was filled with 150 ml of water containing 1 g of sodium chlorite (Alfa Products). To this solution was added 5 ml of 6M H_2SO_4 . The ClO_2 produced was degassed with N_2 from the generator flask into 300 ml of water in a storage flask immersed in an ice-bath. Excess ClO_2 was vented to an aqueous KI solution for decomposition. With this method, stock solutions of 1000 ppm ClO_2 in water were obtained. In analyses and dilution, aliquots of the solution were drawn off from the storage flask by a Teflon stopcock at the bottom of the storage flask.

The ClO_2 concentration was determined by standard iodometric titration.

Preparation and Reaction of Organic Compounds with ClO_2

The organic stock solution was prepared by the addition of 0.2 g of the test material to 200 ml of deionized water buffered at pH 7 with sodium and potassium phosphates. For test chemicals of low water-solubility, a saturated solution was prepared by stirring the test chemical in water for 12 hours and then filtering the solution.

The reaction mixture was prepared by adding 70 ml of the ClO_2 solution to 70 ml of the test chemical stock solution in a glass-stoppered 250-ml Erlenmeyer flask covered with aluminum foil. A control solution was also prepared in which 70 ml of deionized water was substituted for the ClO_2 solution. The solutions were allowed to stand for 17 hours.



LSR-63

FIGURE 1 PREPARATION OF CHLORINE DIOXIDE

After 17 hours, the pH of the solutions was determined with an Orion Model 701 digital pH meter. The final ClO_2 and chlorite (ClO_2^-) concentrations were determined by iodometric difference titration before and after degassing the ClO_2 with nitrogen from the reaction flask. The titration of the degassed solution was considered to be a measure of the ClO_2 concentration.

The concentrations of the test chemicals were monitored by gas chromatography (gc), high-pressure liquid chromatography (hplc), or ultraviolet (uv) spectrophotometry. Specific conditions for these chemicals appear in Appendix A, Effects of Reactions of Ozone in Water, Volume 1.

Gas chromatographic analyses were performed with a Hewlett-Packard Model 5711 gas chromatograph using a flame ionization detector or with a Varian 2100 gas chromatograph equipped with an Analog Technology electron capture detector. Peak areas were determined with a Spectra-Physics "Minigrator" digital integrator.

High-pressure liquid chromatographic analyses were performed with a Spectra-Physics Model 3500B liquid chromatograph equipped with a Model 230 uv detector and a Shoeffel Model FS 870 variable wavelength fluorometer.

Ultraviolet analyses were performed with a Perkin-Elmer Model 200 uv-vis spectrophotometer.

New products arising in gas chromatographic profiles were identified with the aid of an LKB 9000 gas chromatograph/mass spectrometer (gc/ms) equipped with a PDP 12 computer.

Microbiology

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium petri plates containing a trace of histidine, only those cells that revert to histidine independence (his^+) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The

8

his⁺ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased 2- to 100-fold.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.³⁻⁷ In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa⁻) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (bio⁻) and in the repair of uv-induced DNA damage (uvrB⁻). The rfa⁻ mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrB⁻ mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his⁺ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.⁷ In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B₁, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthraces, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4° C on minimal medium plates, supplemented with an excess of biotin and histidine. The plates with the plasmid-carrying strains also contain ampicillin (25 µg/ml) to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every two months from single colony reisolates that were

checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of either the aromatic amino type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.^{6, 8-10} Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.⁸ In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed, sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of MgCl₂ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of H₂O.

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43° C heating block, we add in the following order:

- (1) 2.00 ml of 0.6% agar*
- (2) 0.05 ml of indicator organisms
- (3) up to 0.25 ml of a solution of the test chemical
- (4) 0.50 ml of metabolic activation mixture (optional).

For negative controls, we use steps (1), (2), and (4) (optional) and 0.05 ml of the solvent used for the test chemical. For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3), and (4) (optional).

This mixture is stirred gently and then poured onto minimal agar plates.[†] After the top agar has set, the plates are incubated at 37° C for 2 days. The number of his⁺ revertant colonies is counted and recorded.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.¹¹ When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on the plates.¹²

* 0.6% agar contains 0.05 mM histidine and 0.05 mM biotin.

† Minimal agar plates consist of, per liter, 15 g of agar, 50 g of glucose, 0.2 g MgSO₄·7H₂O, 2 g of citric acid monohydrate, 10 g of K₂HPO₄, and 3.5 g of NaH₂PO₄·4H₂O.

The S. cerevisiae tester strain is stored at -80°C . For each experiment, the tester strain is inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 37°C with aeration.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged, and the cells are resuspended at a concentration of 10^8 cells/ml in a 0.067 M phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.0 ml of the organism
- 0.5 ml of either the metabolic activation mixture or buffer
- 1.0 ml of the test chemical.

Several doses of the chemical (up to 5%, w/v or v/v) are tested in each experiment, and appropriate controls are included.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is diluted serially in sterile physiological saline, and 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on tryptone-yeast agar plates; five plates are used for the 10^{-3} dilution and three plates are used for the 10^{-5} dilution. The plates are incubated for 2 days at 30°C , followed by 2 days at 4°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the 10^{-3} dilution are scanned with a dissecting microscope at 10 X magnification, and the number of red colonies or red sectors (mitotic recombinants) is recorded. The surviving fraction of organisms is determined from the number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

Bacterial Assays in Suspension

The bacterial liquid suspension assay is designed to determine relative toxicity. It employs one or more of the five standard Salmonella tester strains, and differs from the standard plate assay by preincubating the chemical with microorganisms in a sodium phosphate buffer. The procedure is as follows:

To a sterile 16 x 150 mm glass test tube is added:

- (1) 0.8 ml of .067 M sodium phosphate buffer (pH 7.4)
- (2) 0.2 ml of indicator organism
- (3) 0.5 ml of metabolic activation mixture (or 0.5 ml of phosphate buffer)
- (4) 0.5 ml of a solution of the test chemical in water.

The negative control is steps (1), (2), and (3), and 0.5 ml of the solvent only.

The suspension is then incubated on a rolling drum apparatus at 37° C for 2 hours. After 2 hours, the suspension is removed from incubation and plated on complete media.

To determine the total cell population, the suspension is diluted to 10^{-6} and 0.1 ml of the 10^{-5} and 10^{-6} dilutions is plated on nutrient agar complete medium and the plates are incubated for 24 hours.

RESULTS

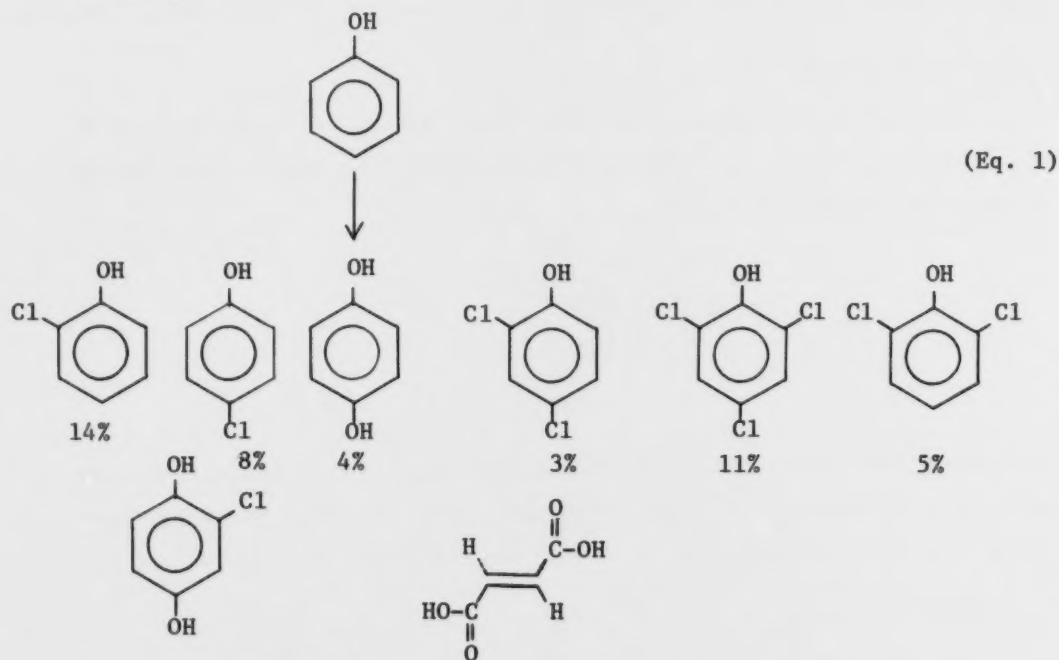
Chemical Results

A listing of the chemicals investigated in this study appears in Table 1 (p. 24) along with their initial concentration, percent reacted, initial and final ClO_2 concentration, and method of analysis.

Of the twenty-six compounds studied, eight compounds were found to be nonreactive with ClO_2 . These compounds are ethanol, acetic acid, caffeine, urea, cholesterol, glycylglycine, coprosterol, and glycerol. The remaining compounds are discussed below.

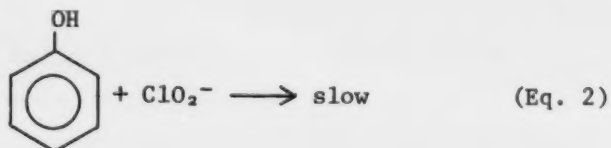
Phenol

The reaction of phenol with ClO_2 produced a variety of oxidation and chloro-substitution products as identified and confirmed by gc/ms techniques.

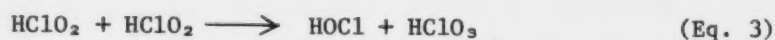


The disappearance of ClO_2 is immediate and complete (2 minutes) as monitored by uv spectrophotometric techniques ($\lambda = 360 \text{ nm}$). The distribution of products is very similar to that observed with hypochlorous acid (HOCl) and phenol.

The rapid disappearance of ClO_2 and the formation of chloro-substitution products indicates the formation of new chlorinating agents. The reactions of chlorite with phenol (Eq. 2) did not proceed at an appreciable rate over a two-day period.



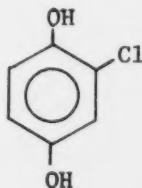
However, chlorite has been reported to disproportionate to form hypochlorous acid and chlorate (Eq. 3).



This reaction may be responsible for the formation of chlorinated phenols.

Hydroquinone

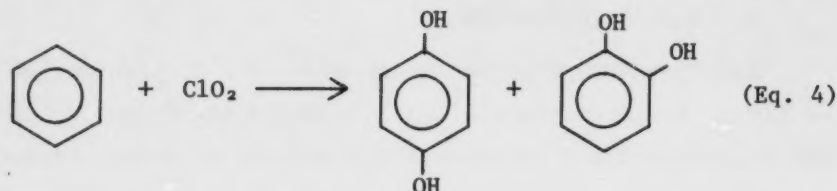
Hydroquinone reacted with ClO_2 to produce four products, one of which was identified as chlorohydroquinone and was also formed in the reaction of phenol with ClO_2 .



The other reaction products form trimethylsilyl (TMS) derivatives and have mass-to-energy (m/e) ratios of 182, 266, and 268, respectively. These products, however, did not contain chlorine.

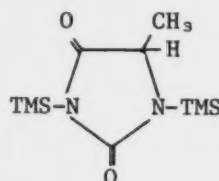
Benzene

The reaction of benzene with ClO_2 produced hydroquinone and catechol in low yield as the only observed products (Eq. 4).

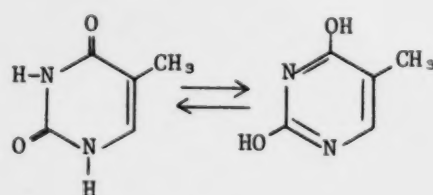


Thymine

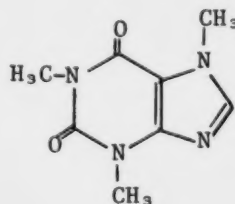
Thymine appeared very reactive with ClO_2 , as indicated by the disappearance of the yellow ClO_2 color within 30 minutes. Three products with m/e ratios of 208, 258, and 268 were detected as trimethylsilyl derivatives. A structure for the $m/e = 258$ component is proposed to be



It is interesting to note that thymine is reactive with ClO_2 but that caffeine is not. This may be the result of the ability of thymine to enolize to a phenol-type structure, which is not possible with caffeine.



Thymine
(Eq. 5)



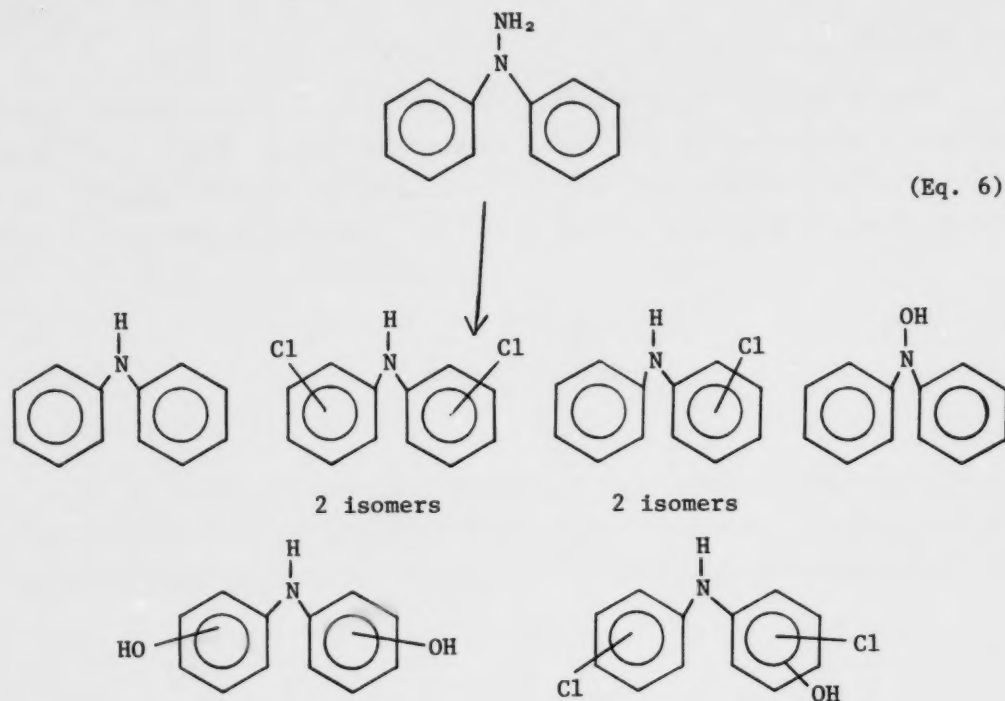
Caffeine

Benzidine

Benzidine reacted readily with ClO_2 at pH 2 (benzidine was used as the hydrochloride salt); however, no new products were observed in gc profiles.

1,1-Diphenylhydrazine

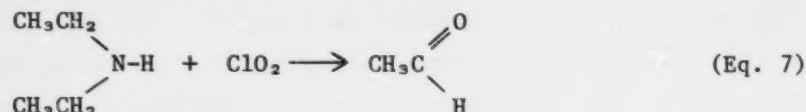
1,1-Diphenylhydrazine solutions turned greyish-red on the addition of ClO_2 . The mass spectral data indicate the formation of diphenylamine and chlorinated and oxygenated derivatives of diphenylamine.



The primary amine nitrogen in 1,1-diphenylhydrazine was thought to be converted to hydroxylamine. However, tests for the presence of hydroxylamine were negative.

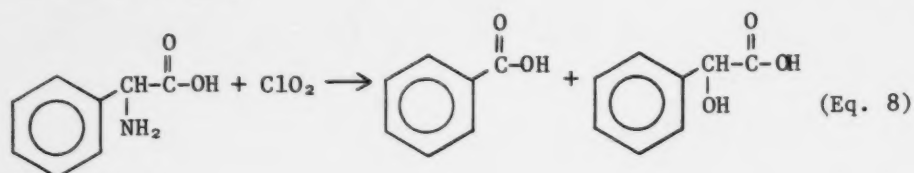
Diethylamine

The reaction of diethylamine with ClO_2 was found to yield acetaldehyde as the only observable product.



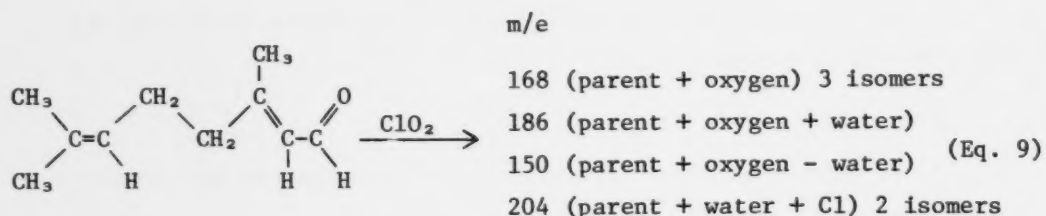
Phenylalanine

Phenylalanine reacted with ClO_2 to produce phenyllactic acid and benzoic acid in low yield.



Citral

The terpenoid citral reacted extensively with ClO_2 , giving rise to many addition and oxidation products. Mass spectral data indicated chlorine incorporation, giving rise to two isomers. These data also indicated the following elemental incorporations:



Cysteine

Cysteine was very reactive with ClO_2 . No new products were observed in gc profiles.

Dibutylphthalate

Dibutylphthalate reacted with ClO_2 , but no new products appeared in gc profiles.

Humic Acids

Humic acid reacted quite well with ClO_2 . A sonicated suspension of humic acids was found to lighten in color when ClO_2 was added, and complete solution was achieved on standing overnight. Total organic carbon levels in solution increased by 30%, indicating that solution was being effected. No products were observed in ether extracts that were subjected to trimethylsilylation. A hexane extract showed no chloroform production by gc analysis using electron capture detection.

Aldrin

Aldrin showed an 81% reaction with ClO_2 . No new products were observed by gc with electron capture detection.

Nitrilotriacetic acid (NTA)

NTA reacted to the extent of 34%. Since no mutagenicity was observed, no attempts were made to investigate products.

Benzoic Acid

Benzoic acid reacted to a slight degree with ClO_2 . However, no new products were observed in gc profiles.

Benzo(a)pyrene

No new products were observed when hplc profiles were obtained using fluorescence detection.

Oleic Acid

Oleic acid reacted readily with ClO_2 . However, no new products were observed in gc profiles.

Glucose

Glucose reacted slowly with ClO_2 . No new products were observed in gc profiles.

Microbiological Results

At the doses tested in the S. typhimurium or S. cerevisiae assays, we found none of the 26 compounds to be mutagenic or recombinogenic, either before or after treatment with chlorine dioxide and either in the

absence or in the presence of metabolic activation. A preliminary experiment (blank control) was conducted with each assay system to determine the potential mutagenic and recombinogenic effects of residual chlorine dioxide. The concentrations of residual chlorine dioxide in these experiments was greater than the residual concentration of chlorine dioxide present in any of the experiments with the selected organic compounds. No mutagenic, recombinogenic, or toxic effects were observed (Tables 2 and 3). The actual sets of data on the 26 compounds appear in Tables 4 through 122. The results of the mutagenic positive controls used in the S. cerevisiae assays appear in Table 123. The maximum micromoles per plate used for each of the substances in the Salmonella/microsome assay is presented in Table 124.

Although we found no mutagenic activity with any of the compounds, other biological information was obtained on some of the compounds, as discussed below.

Phenol

In the assays with S. typhimurium, phenol was toxic after treatment with chlorine dioxide. In three experiments in which 67% (Table 18), 48% (Table 20), and 82% (Table 21) of the phenol reacted with the chlorine dioxide, toxicity was observed at all doses above 3.3 g/plate when tested without metabolic activation. In these three experiments, when metabolic activation was incorporated into the assay, the toxicity was reduced. When only 32% of the phenol reacted with chlorine dioxide (Table 19), less toxicity was seen than in the other three assays. Thus, it appears that the products produced by the degradation of phenol by chlorine dioxide are toxic to S. typhimurium.

In one of the three assays with S. cerevisiae, phenol appeared to be toxic when tested after treatment. In one assay (Table 23), 63% of the phenol remained after treatment. No toxicity was observed in the test even though the doses used were about double those used in the other two tests. In one assay in which no toxicity was observed (Table 24), 32% of the phenol remained after treatment; the highest concentration used in this test was 0.0055%. In the test in which 33% of the phenol remained after chlorine dioxide treatment (Table 22), toxicity was observed at a concentration of 0.007%. It appears that the more extensive degradation of phenol by chlorine dioxide produces products that were toxic to S. cerevisiae.

Diethylamine

In one of the two assays with S. cerevisiae, diethylamine appeared to be toxic at the highest dose tested (7% survival; Table 38) after treatment with chlorine dioxide. In this assay, 17% of the diethylamine reacted with chlorine dioxide. In the assay showing no toxicity (Table 37), 34% of the diethylamine reacted with chlorine dioxide. Thus, it appears that the products formed by the reaction may be toxic. Acetaldehyde is the only product observed from the reaction of diethylamine with chlorine dioxide. When actaldehyde was tested with S. cerevisiae, it was not toxic at concentrations higher than these of this assay (Eckford and Simmon, unpublished data). The toxicity may be due to other unidentified products or to random variables inherent in the test.

Hydroquinone

When tested with S. typhimurium (Tables 39 through 43), some toxicity was observed at the highest dose of hydroquinone tested before treatment with chlorine dioxide (197 µg/plate) but not at the next lowest dose (192 µg/plate). However, when the metabolic activation preparation was incorporated into the assay, no toxicity was observed. After treatment with chlorine dioxide, hydroquinone was toxic without metabolic activation at all doses tested (16-102 µg/plate). With metabolic activation, toxic results were seen at doses of 82 µg/plate and above in one of the assays.

When tested with S. cerevisiae (Tables 41 through 43), hydroquinone was not toxic before treatment with chlorine dioxide. After treatment, hydroquinone was toxic with and without metabolic activation at doses above 0.0016% concentration.

Caffeine

In one of two assays with S. cerevisiae (Tables 46 and 47), caffeine appeared to be toxic at the highest dose (0.0194% concentration) when tested after treatment with chlorine dioxide. The chemical analysis indicated that the concentration of caffeine was approximately the same in both experiments and that caffeine did not react with chlorine dioxide.

The experiment in which caffeine appeared to be toxic was performed on the same day as the one assay in which diethylamine appeared to be toxic. Thus, for caffeine, as for diethylamine, the toxicity may be due to random variables inherent in the performance of the test.

Benzidine

When benzidine was 100% reacted with chlorine dioxide, toxicity was observed in both of the microbiological assays. In assays with Salmonella (Tables 48 and 49), the product(s) from the reacted benzidine were toxic at all doses above 0.05 ml when the metabolic activation system was not incorporated into the assay. With metabolic activation, the product(s) were less toxic. In the Saccharomyces assays (Tables 50 and 51), toxicity was observed at all doses tested without metabolic activation (2% survival at the lowest doses) and on all but the lowest dose tested with metabolic activation.

Benzidine was not toxic at any dose tested when it had not been 100% reacted with chlorine dioxide. Thus, it appears that the products formed from the treatment of benzidine with chlorine dioxide are toxic to S. cerevisiae and S. typhimurium.

The results with chlorine dioxide are in contrast to those with ozone (draft report entitled "I. The Effects of Ozonation Reactions in Water," submitted to EPA in February 1977). In the latter case, benzidine was transformed from a promutagen, procarcinogen to a form that increased mitotic recombination in S. cerevisiae and mutation in S. typhimurium in the absence of metabolic activation.

Aldrin

In the first assay with S. cerevisiae (Table 97), the numbers of mitotic recombinants per milliliter and per 10^5 survivors were elevated. However, the increases did not appear to be dose-related. After further testing with the same doses, it was concluded that aldrin was not mutagenic in S. cerevisiae at the doses tested (Tables 97 and 98).

Dibutylphthalate

Dibutylphthalate appeared toxic in one of three assays with S. cerevisiae (Tables 106 through 108) after treatment with chlorine dioxide. Toxicity was observed at a concentration of 0.0007% and higher (Table 106). In this experiment, 36% of the dibutylphthalate remained after chlorine dioxide treatment. In two other experiments in which 58% (Table 107) and 45% (Table 108) of the dibutylphthalate remained after chlorine dioxide treatment, toxicity was not observed at a concentration of 0.00076%. Thus, it appears that the more extensive degradation of dibutylphthalate by chlorine dioxide produced products that were toxic to S. cerevisiae.

Coprosterol

In one assay with S. cerevisiae, coprosterol was toxic after treatment with chlorine dioxide. Coprosterol was nonreactive with chlorine dioxide. Upon further testing, using the same doses, coprosterol did not appear to be toxic to S. cerevisiae (Tables 117 and 118).

DISCUSSION

The chemical results indicate the chlorine dioxide will oxidize a wide variety of organic molecules. With the exception of selected cases (such as phenol, hydroquinone, and citral) the production of chlorine-containing organics appeared to be minimal. In those cases where chlorine incorporation was observed, the reaction conditions (high substrate-low oxidant) have to be considered to understand the significance of this finding. Glabisz¹³ has found that as the oxidant to substrate ratio increases, the amount of chlorinated products decreases in the case of the reaction of chlorine dioxide with phenol. This could indicate that chlorinated products readily undergo further reaction in the presence of chlorine dioxide or that competitive oxidative mechanisms result as a function of the oxidant/substrate ratio. Since the objective of this report was to generate intermediate products (low oxidant/substrate ratio), the oxidant/substrate ratio should be considered in any extrapolation of the biological results to actual use conditions.

The results of the microbial mutagenicity assays indicated that none of the products from chlorine dioxide treatment of these selected compounds are potent mutagens. Although this result is reassuring, it must be tempered with the realization that most of the chemicals were tested at extremely low concentrations. The maximum volume of water containing potential mutagens (post-chlorine dioxide treatment) was 0.25 ml in the Salmonella assay and 1.0 ml in the Saccharomyces assay. Most mutagens are detectable in the 10- to 1000- μ g range in the Salmonella assay³ and 0.5 to 5% in the Saccharomyces assay (Simmon, in press). Therefore, 40 to 4000 ppm of a typical mutagen would have been required to elicit a response in Salmonella, and 10,000 to 100,000 ppm would have been required for the Saccharomyces assay. However, potent mutagens may be detected in the nanogram range in Salmonella (<1 ppm) and in the low ppm range by Saccharomyces. Thus, the absence of any mutagenic activity in this study indicates that no potent mutagens were formed.

Our experience indicates that we probably would not have detected volatile mutagens in the Salmonella assay if they were produced by ClO_2 treatment.¹ Many alkyl halides can only be detected when the Salmonella are exposed to them in a sealed container, such as a desiccator. This applies to compounds with boiling points in excess of 100°C .

Bacterial assays in suspension, with S. typhimurium strain TA100 were performed on ClO_2 , NaClO_2^- , ClO_3^- , and NH_2Cl to determine toxicity. (Tables 124 through 127). No toxicity was observed with ClNH_2 and NaClO_3 when tested at concentrations of 5000 ppm and 4529 ppm, respectively. NaClO_2 was toxic at doses of 2850 ppm and above and ClO_2 was toxic at doses of 19 ppm and above.

EXECUTIVE SUMMARY FOR VOLUMES 1 AND 2

The use of rapid microbial bioassays to assess the potential mutagenic effect of disinfectant chemicals on selected organics is subject to several limitations. Therefore, when drawing conclusions from the obtained data, the following points should be considered.

First, the sensitivity of the screening procedure requires relatively high concentrations of the intermediates or final products. To achieve these concentrations, attempts were made to achieve 50% decomposition levels of the selected organic compound. This necessitated the use of high substrate-to-oxidant concentration ratios to ensure the existence of intermediate products at concentrations high enough to be tested. This condition, which is opposite to that normally observed in water-treatment plants (where the substrate-to-oxidant ratios are very low), creates two problems. 1) There is a greater potential for artifact formation, such as coupling reactions between intermediates (e.g., 4,4'-dihydroxybiphenyl in the oxidation of phenol). 2) Changes in reaction mechanisms are possible, leading to products that may not exist under actual treatment conditions (such as the chlorophenols from the reaction of phenol with chlorine dioxide).

The formation of concentrates by extraction and concentration is one solution to the above problem, and this technique can be applied under actual disinfection conditions. However, concentration steps should be employed judiciously to avoid loss of volatiles, prevent condensation reactions, and prevent the decomposition of reactive intermediates. Also, extraction is only effective for those compounds with high partition coefficients for the extraction solvent. Thus, highly water-soluble compounds (such as hydroperoxides and hydroxyhydroperoxides), which might be expected from oxidation reactions, would be missed in the screening procedure.

In some cases, extraction methods have to be employed due to the solubility of the organic compound in water. This is particularly true in the case of chlorinated hydrocarbons and pesticides, where solubilities never exceed the low ppb range.

Another possibility for evaluating mutagenicity is a material balance of the substrate and its transformation products. This requires that all the intermediate products be chemically identified, quantified, and screened individually for mutagenic activity. In many cases this is a difficult task, and often one has to settle for partial identifications that will not completely define the mixture.

Mutagenic screening of the individual components can create a ranking of mutagenic potential for the entire mixture. The mutagenic potential is defined as the number of revertants per microgram tested times the percentage of the compound in the mixture.

$$\text{Mutagenic potential} = \frac{\text{revertants}}{\mu\text{g}} \times \text{weight \% in mixture}$$

Using this ranking system, the contribution of each component of the total mutagenicity of the sample can be evaluated. This method requires the procurement of pure chemicals, many of which may not be commercially available. However, it allows the testing of each compound at high concentration levels where effects are more likely to be observed rather than in systems where concentrations are below an observable effect level (actual mixture).

REFERENCES

1. V. F. Simmon, K. Kauhanen, and R. G. Tardiff. Mutagenic activity of chemicals identified in drinking water. *In Progress in Genetic Toxicology* (D. Scott, B. A. Bridges, and F. H. Sobels, eds). Elsevier/North-Holland Biomedical Press, Amsterdam, 1977, pp. 249-258.
2. J. J. Rook. Formation of haloforms during chlorination of natural waters. *Water Treatment and Examination* 23, Part 2, 234 (1974).
3. J. McCann, E. Choi, E. Yamasaki, and B. N. Ames. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc. Nat. Acad. Sci. USA* 72, 5135-5139 (1975).
4. B. N. Ames, E. G. Gurney, J. A. Miller, and H. Bartsch. Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. *Proc. Nat. Acad. Sci. USA* 69, 3128-3132 (1972).
5. B. N. Ames, F. D. Lee, and W. E. Durston. An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc. Nat. Acad. Sci. USA* 70, 782-786 (1973).
6. B. N. Ames, W. E. Durston, E. Yamasaki, and F. D. Lee. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Nat. Acad. Sci. USA* 70, 2281-2285 (1973).
7. J. McCann, N. E. Spingarn, J. Kobori, and B. N. Ames. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. *Proc. Nat. Acad. Sci. USA* 72, 979-983 (1975).
8. L. A. Poirier and V. F. Simmon. Mutagenic-carcinogenic relationships and the role of mutagenic screening tests for carcinogenicity. *Clin. Toxicol.* 9, 761-771 (1976).
9. L. D. Kier, E. Yamasaki, and B. N. Ames. Detection of mutagenic activity in cigarette smoke condensates. *Proc. Nat. Acad. Sci. USA* 71, 4159-4163 (1974).
10. B. N. Ames, J. McCann and E. Yamasaki. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome carcinogenicity test. *Mutation Res.* 31, 347-364 (1975).

11. F. K. Zimmermann and R. Schwaier. Induction of mitotic gene conversion with nitrous acid, 1-methyl-3-nitro-1-nitrosoguanidine and other alkylating agents in Saccharomyces cerevisiae. Mol. Gen. Genet. 100, 63-69 (1967).
12. D. J. Brusick and V. W. Mayer. New developments in mutagenicity screening techniques with yeast. Environ. Health Perspectives 6, 83-96 (1973).
13. U. Glabisz. Über die Wirkung von Chlordioxid auf einwertige Phenole. Chem. Techn. 19 (c), 352-356 (1967).

Table 1
CHEMICALS AND RESULTS OF CHLORINE DIOXIDE INVESTIGATIONS

Compound	Initial Concentration (ppm)	% Reacted	Initial ClO ₂ Concentration (ppm)	pH		Method of Analysis	mmoles Organic	mmoles ClO ₂	Contact Time (hours)
				Initial	Final				
Ethanol	520	0	125	7.0	7.0	G-C	11.3	1.8	17
Acetic acid	566	0	135	6.3	6.7	G-C	9.4	2.2	16
NTA	395	34	135	6.9	6.7	uv Zirconium Complex	1.9	2.0	16
Phenol	451	43	290	6.9	6.2	G-C	4.3	4.3	17
1,1-Diphenylhydrazine	193	41	135	6.9	6.9	G-C	1.0	2.0	16.
Benzoic acid	557	10	356	7.0	6.9	G-C	4.5	4.3	16
Diethylamine	571	34	337	7.6	7.2	G-C	7.3	5.0	17
Hydroquinone	769	53	297	6.9	6.0	G-C	7.0	4.4	17
Caffeine	434	0	337	7.0	7.0	G-C	2.5	5.0	17
Benzidine	136	59	43	2.0	2.0	G-C	0.72	0.71	17
Urea	574	0	337	7.0	6.3	G-C	9.5	5.0	17
Benzene	221	21	125	7.0	6.7	G-C	2.3	1.8	18
Oleic acid	21	33	573	7.0	6.3	G-C	0.07	7.9	17
Humic acid	105*	--	303	7.0	7.0	--	--	4.5	18
Glucose	510	24	100	7.0	7.0	G-C	2.3	1.5	17
Cholesterol	0.013	0	1.0	7.0	7.0	G-C	3.3x10 ⁻³	0.015	17
Thymine	331	32	99	7.0	6.9	G-C	2.6	1.5	17
Benzo(a)pyrene		12	1.0	7.0	7.0	HPLC Fluorescence Det.	6.7x10 ⁻⁶	0.015	17
Cysteine	471	97	99	7.0	7.0	G-C	3.8	1.5	17
Phenylalanine	326	94	99	7.0	7.0	G-C	2.1	1.5	17
Aldrin	0.003	31	2.0	7.0	7.0	G-C	9.2x10 ⁻⁶	0.029	13
Glycylglycine	596	0	297	7.0	6.7	uv	4.0	4.4	17
Dibutylphthalate	42	55	232	7.0	7.0	G-C	0.15	4.2	17

* Suspension.

Table 1 (concluded)

Compound	Initial Concentration (ppm)	% Reacted	Initial ClO ₂ Concentration (ppm)	pH		Method of Analysis	mmoles Organic	mmoles ClO ₂	Contact Time (hours)
				Initial	Final				
Citral	405	63	173	7.0	7.0	G-C	2.7	2.6	18
Coprosterol	0.003	0	2.0	7.0	7.0	G-C	2.0x10 ⁻³	0.029	17
Glycerol	439	0	135	7.0	7.0	G-C	5.3	2.0	17

Table 2
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - BLANK CONTROL
EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		35	9	12	34 138
	+		21	10	18	30 152
Positive controls						
β-Propiolactone	-	50	490	666	1678	185 770
9-Aminoacridine	-	100				46 180
2-Nitrofluorene	-	50				1280 2067
AF2	-	0.1				
2-Anthramine	-	10	35	24	18	
	+	10	620	10	1849	
Post-treatment						
Blank control	-	0.06	24	11	13	31 146
	-	0.11	22	10	13	18 131
	-	0.29	24	13	16	33 135
	-	0.57	17	8	15	31 114
	-	1.14	20	5	15	35 129
	-	2.85	27	5	10	18 148
	+	0.06	27	11	31	24 142
	+	0.11	15	14	14	33 158
	+	0.29	11	16	19	35 124
	+	0.57	21	6	23	33 125
	+	1.14	13	8	14	18 120
	+	2.85	12	10	18	20 108

Table 3

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BLANK CONTROL
EXPERIMENT 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-5}$)	Per 10^5 Survivors
Negative control	-		6.8	100	9.0	13
	+		5.6	100	4.0	7.1
Post-treatment Blank control	-	0.0005	6.3	93	3.0	4.5
	-	0.0002	6.2	91	5.0	8.1
	-	0.0001	6.9	101	4.0	5.8
	-	0.00005	8.1	119	3.0	3.7
	-	0.00001	5.6	82	2.0	3.6
	+	0.0005	7.2	129	2.0	2.8
	+	0.0002	6.0	107	3.0	5.0
	+	0.0001	7.8	139	5.0	6.4
	+	0.00005	7.8	139	1.0	1.3
	+	0.00001	6.6	118	11	17

Table 4

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - ETHANOL
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		24	8	11	17
	+		11	11	20	27
Positive controls						
β-Propiolactone	-	10	345			
9-Aminoacridine	-	100		954		
2-Nitrofluorene	-	50			1364	
AF2	-	0.1				996
2-Anthramine	-	2.5	20	9	25	26
	+	2.5	221	68	935	708
Pre-treatment Ethanol	-	55.3	18	7	6	17
	+	55.3	12	10	12	19
Post-treatment Ethanol	-	9.4	27	14	8	22
	-	18.8	18	8	11	14
	-	28.2	26	6	7	16
	-	37.6	19	7	10	13
	-	47.0	17	11	12	26
	+	9.4	13	3	13	26
	+	18.8	14	9	13	28
	+	28.2	12	8	19	25
	+	37.6	4	11	17	25
	+	47.0	9	10	12	18

Table 5

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - ETHANOL

EXPERIMENT 8

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98
Negative control	-		17	11	14	30
	+		18	9	18	34
Positive controls						
Sodium azide	-	1.0	371	1853	1162	506
9-Aminoacridine	-	100				35
2-Nitrofluorene	-	50				1529
AF2	-	0.1	17	16	13	
2-Anthramine	-	2.5	153	143	1334	
	+	2.5				
Pre-treatment Ethanol	-	133	26	19	10	16
	+	133	25	13	19	33
Post-treatment Ethanol	-	27	21	13	14	20
	-	53	21	8	10	15
	-	80	16	13	10	20
	-	106	16	10	11	21
	-	133	23	9	14	34
	+	27	26	12	12	28
	+	53	12	12	14	23
	+	80	12	13	13	19
	+	106	15	19	16	24
	+	133	17	16	13	25

Table 6
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - ETHANOL
EXPERIMENT 17

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate	
			TA100	
Negative control	-		159	
	+		133	
Positive controls 2-Anthramine	-	2.5	266	
	+	2.5	1431	
Pre-treatment Ethanol	-	130	88	
	+	130	120	
Post-treatment Ethanol	-	26	139	
	-	52	134	
	-	78	134	
	-	104	136	
	-	130	175	
	+	26	119	
	+	52	122	
	+	78	135	
	+	104	121	
	+	130	164	

Table 7

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - ETHANOL
EXPERIMENT 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.7	100	2.0	3.5
	+		5.5	100	7.0	13
Pre-treatment Ethanol	-	0.0088	6.0	105	6.0	10
	+	0.0088	6.4	116	12	19
Post-treatment Ethanol	-	0.0075	5.9	104	10	17
	-	0.0038	7.0	123	6.0	8.6
	-	0.0019	5.6	98	4.0	7.1
	-	0.0008	7.0	123	8.0	11
	+	0.0075	6.5	118	5.0	7.7
	+	0.0038	5.9	107	5.0	8.5
	+	0.0019	6.5	118	6.0	9.2
	+	0.0008	6.6	120	6.0	9.1

Table 8

IN VITRO ASSAYS WITH *SACCHAROMYCES CEREVISIAE* - ETHANOL
EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.6	100	3.0	4.5
	+		6.7	100	4.0	6.0
Pre-treatment Ethanol	-	0.021	8.2	124	6.0	7.3
	+	0.021	7.1	106	1.0	1.4
Post-treatment Ethanol	-	0.021	5.0	76	7.0	14
	-	0.011	6.7	102	1.7	2.5
	-	0.0053	8.6	130	6.0	7.0
	-	0.0021	8.6	130	6.0	7.0
	+	0.021	6.2	93	7.0	11
	+	0.011	7.3	109	8.0	11
	+	0.0053	8.8	131	4.0	4.5
	+	0.0021	9.6	143	3.0	3.1

Table 9

IN VITRO ASSAYS WITH *SALMONELLA* TYPHIMURIUM - ACETIC ACID
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		24	8	11	17
	+		11	11	20	27
Positive controls						
β-Propiolactone	-	10	345			
9-Aminoacridine	-	100		954		
2-Nitrofluorene	-	50			1364	
AF2	-	0.1				996
2-Anthramine	-	2.5	20	9	25	26
	+	2.5	221	68	935	708
Pre-treatment	-	204	13	9	14	13
Acetic acid	+	204	15	15	19	24
						97
						105
Post-treatment	-	37.4	20	8	8	17
Acetic acid	-	74.9	24	5	16	18
	-	112.3	16	7	9	27
	-	149.8	16	5	11	19
	-	187.2	20	5	9	18
						113
	+	37.4	17	11	15	14
	+	74.9	10	11	13	28
	+	112.3	21	10	20	31
	+	149.8	9	10	7	23
	+	187.2	8	12	16	22
						100
						87
						104
						86
						112

Table 10

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - ACETIC ACID

EXPERIMENT 8

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	11	14	30
	+		18	9	18	34
Positive controls						
Sodium azide	-	1.0	371	1853		
9-Aminoacridine	-	100			1162	
2-Nitrofluorene	-	50				506
AF2	-	0.1				998
2-Anthramine	-	2.5	17	16	13	35
	+	2.5	153	143	1334	1529
						1602
Pre-treatment	-	141.5	25	8	17	32
Acetic acid	+	141.5	17	8	21	26
						95
Post-treatment	-	28.3	26	8	11	33
Acetic acid	-	56.6	15	5	13	28
	-	84.9	20	8	10	35
	-	113.2	24	13	15	35
	-	141.5	25	10	21	40
						142
	+	28.3	17	8	18	38
	+	56.6	18	9	14	34
	+	84.9	21	13	10	39
	+	113.2	16	15	22	37
	+	141.5	22	12	18	43
						129
						112
						104
						122
						153

Table 11

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - ACETIC ACID

EXPERIMENT 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10 ⁻⁷)	Percent	Per ml (x 10 ⁻³)	Per 10 ³ Survivors
Negative control	-		5.7	100	2.0	3.5
	+		5.5	100	7.0	13
Pre-treatment Acetic acid	-	0.033	3.9	68	4.0	10
	+	0.033	5.8	105	3.0	5.2
Post-treatment Acetic acid	-	0.030	4.8	84	4.0	8.3
	-	0.015	6.9	121	6.3	9.1
	-	0.008	6.2	109	6.0	9.7
	-	0.003	6.4	112	5.0	7.8
	+	0.030	5.4	98	5.0	9.3
	+	0.015	6.4	116	2.0	3.1
	+	0.008	6.8	124	5.0	7.4
	+	0.003	7.8	142	2.5	3.2

Table 12

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - ACETIC ACID

EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.6	100	3.0	4.5
	+		6.7	100	4.0	6.0
Pre-treatment Acetic acid	-	0.023	7.6	115	6.0	7.9
	+	0.023	6.2	93	6.0	9.7
Post-treatment Acetic acid	-	0.023	5.5	83	3.0	5.5
	-	0.011	9.2	139	7.0	7.6
	-	0.006	6.0	91	6.0	10
	-	0.002	8.0	121	5.0	6.3
	+	0.023	4.9	73	6.0	12
	+	0.011	6.3	94	2.0	3.2
	+	0.006	6.8	101	2.0	2.9
	+	0.002	6.4	96	6.0	9.4

Table 13

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - ACETIC ACID
EXPERIMENT 10

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	1.0	1.9
	+		3.5	100	1.0	2.9
Pre-treatment Acetic acid	-	0.021	5.0	93	1.0	2.0
	+	0.021	4.4	126	2.0	4.5
Post-treatment Acetic acid	-	0.021	5.4	100	4.0	7.4
	-	0.010	5.2	96	<1.0	1.9
	-	0.005	3.9	72	2.0	5.1
	-	0.002	2.7	50	<1.0	3.7
	+	0.021	3.9	111	<1.0	2.6
	+	0.010	4.5	129	3.0	6.7
	+	0.005	4.4	126	<1.0	2.3
	+	0.002	4.1	117	3.0	7.3

Table 14

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - NITRILOTRIACETIC ACID
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		24	8	11	17
	+		11	11	20	27
Positive controls						
β-Propiolactone	-	10	345			
9-Aminoacridine	-	100		954		
2-Nitrofluorene	-	50			1364	
AF2	-	0.1				996
2-Anthramine	-	2.5	20	9	25	26
	+	2.5	221	68	935	708
Pre-treatment						
Nitrilotriacetic acid	-	131.3	31	8	11	13
	+	131.3	10	10	17	32
Post-treatment						
Nitrilotriacetic acid	-	7.1	19	1	6	18
	-	14.2	23	10	9	18
	-	21.3	28	6	9	18
	-	28.4	21	8	11	23
	-	35.5	27	11	12	12
	+	7.1	13	13	13	15
	+	14.2	14	9	22	22
	+	21.3	18	10	16	22
	+	28.4	7	9	11	19
	+	35.5	15	8	17	24

Table 15

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - NITRILOTRIACETIC ACID
EXPERIMENT 8

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TAL535	TAL537	TAL538	TA98	TAL100
Negative control	-		17	11	14	30	120
	+		18	9	18	34	147
Positive controls							
Sodium azide	-	1.0	371	1853			
9-Aminoacridine	-	100			1162		
2-Nitrofluorene	-	50				506	998
AF2	-	0.1	17	16	13	35	153
2-Anthramine	-	2.5	153	143	1334	1529	1602
	+	2.5					
Pre-treatment	-	99	25	15	14	20	127
Nitrilotriacetic acid	+	99	16	9	11	28	104
Post-treatment	-	13	25	5	17	21	140
Nitrilotriacetic acid	-	26	15	17	16	19	119
	-	39	22	11	11	26	120
	-	52	25	5	14	25	106
	-	65	29	17	11	22	131
	+	13	17	7	20	18	125
	+	26	23	6	24	37	140
	+	39	13	7	12	31	140
	+	52	16	14	23	32	137
	+	65	12	12	11	35	125

Table 16

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - NITRILOTRIACETIC ACID
EXPERIMENT 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-5}$)	Per 10^5 Survivors
Negative control	-		5.7	100	2.0	3.5
	+		5.5	100	7.0	13
Pre-treatment Nitriлотriacetic acid	-	0.0210	6.4	112	10.0	16
	+	0.0210	5.6	102	7.0	13
Post-treatment Nitriлотriacetic acid	-	0.0057	6.8	119	6.0	8.8
	-	0.0029	5.9	104	8.0	14
	-	0.0014	3.6	63	6.0	17
	-	0.0006	6.9	121	3.0	4.3
	+	0.0057	6.7	122	5.0	7.4
	+	0.0029	5.9	107	8.0	14
	+	0.0014	6.2	113	10	16
	+	0.0006	6.7	122	2.5	3.7

Table 17

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - NITRILOTRIACETIC ACID
EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		6.6	100	3.0	4.5
	+		6.7	100	4.0	6.0
Pre-treatment Nitriлотriacetic acid	-	0.0158	6.4	97	5.0	7.8
	+	0.0158	5.3	79	4.0	7.5
Post-treatment Nitriлотriacetic acid	-	0.0104	6.6	100	5.0	7.6
	-	0.0052	6.1	92	1.0	8.2
	-	0.0026	7.3	111	2.0	2.7
	-	0.0010	5.4	82	8.0	15
	+	0.0104	6.3	94	3.0	4.8
	+	0.0052	5.8	87	3.0	5.2
	+	0.0026	5.3	79	5.0	9.4
	+	0.0010	6.2	93	1.0	1.6

Table 18

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - PHENOL
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		24	8	11	17 125
	+		11	11	20	27 107
Positive controls						
β-Propiolactone	-	10	345			
9-Aminoacridine	-	100		954		
2-Nitrofluorene	-	50			1364	
AF2	-	0.1				996
2-Anthramine	-	2.5	20	9	25	26 125
	+	2.5	221	68	935	708 1235
Pre-treatment	-	134	17	10	11	17 101
Phenol	+	134	12	7	16	23 100
Post-treatment	-	9	T*	T	T	T 52
Phenol	-	18	T	T	T	T T
	-	26	T	T	T	T T
	-	35	T	T	T	T T
	-	44	T	T	T	T T
	+	9	11	6	27	29 149
	+	18	11	8	12	23 143
	+	26	15	7	10	28 173
	+	35	16	8	17	30 187
	+	44	T	T	T	T 66

* T, toxic.

Table 19
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - PHENOL
EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	- +		13 8	3 4	13 67	23 57	99 136
Positive controls							
β-Propiolactone	-	10	146				
9-Aminoacridine	-	100		630			
2-Nitrofluorene	-	10			350		
AF2	-	0.1					974
2-Anthramine	-	2.5	21	3	15	337	162
	+	2.5	34	32	233	310	490
Pre-treatment Phenol	-	108	10	3	5	16	80
	+	108	12	5	59	20	136
Post-treatment Phenol	-	15	14	11	12	25	101
	-	30	15	7	12	45	166
	-	44	6	4	9	17	215
	-	59	6	0	4	18	171
	-	74	1	5	4	1	76
	+	15	13	6	17	15	115
	+	30	8	4	17	31	125
	+	44	14	4	20	30	111
	+	59	8	5	22	27	128
	+	74	6	7	22	33	169

Table 20^{*}
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - PHENOL
EXPERIMENT 12

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TAL535	TAL537	TAL538	TA98	TA100
Negative control	-		17	5	8	32	112
	+		10	6	9	32	85
Positive controls Sodium azide 9-Aminoacridine 2-Nitrofluorene AF2 2-Anthramine	-	1.0	360				
	-	100		1675			
	-	20			654		
	-	0.1				239	694
	-	2.5	19	5	12	42	145
	+	2.5	149	153	806	1176	1704
Pre-treatment Phenol	-	113	15	5	8	22	83
	+	113	6	14	11	24	101
Post-treatment Phenol	-	12	T*	T	T	T	T
	-	23	T	T	T	T	T
	-	35	T	T	T	T	T
	-	47	T	T	T	T	T
	-	59	T	T	T	T	T
	+	12	7	8	4	27	117
	+	23	10	6	8	39	129
	+	35	4	4	8	45	134
	+	47	8	5	7	34	119
	+	59	9	5	10	31	132

* T, toxic.

Table 21
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - PHENOL
EXPERIMENT 17

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		24	8	13	25 159
	+		13	8	23	38 133
Positive controls						
Sodium azide	-	1.0	912	2071	1520	46
9-Aminoacridine	-	100				266
2-Nitrofluorene	-	50				1431
AF2	-	0.1	17	11	16	31
2-Anthramine	-	2.5	391	30	454	1718
	+	2.5				
Pre-treatment	-	200	30	7	8	24 137
Phenol	+	200	8	11	22	31 122
Post-treatment	-	7	24	10	12	39 144
Phenol	-	14	T*	T	T	T T
	-	22	T	T	T	T T
	-	29	T	T	T	T T
	-	36	T	T	T	T T
	+	7	14	6	18	24 154
	+	14	14	9	13	31 200
	+	22	16	11	17	20 296
	+	29	13	13	19	29 302
	+	36	T	T	T	41 T

* T, toxic.

Table 22

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - PHENOL
EXPERIMENT 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.7	100	2.0	3.5
	+		5.5	100	7.0	13
Pre-treatment Phenol	-	0.0214	7.6	133	6.0	7.9
	+	0.0214	7.7	140	12	16
Post-treatment Phenol	-	0.0070	T*	T	T	T
	-	0.0035	3.2	56	9.0	28
	-	0.0018	7.2	126	10	14
	-	0.0007	6.0	105	3.0	5.0
	+	0.0070	T	T	T	T
	+	0.0035	2.6	47	14	54
	+	0.0018	8.8	160	5.0	5.7
	+	0.0007	7.7	140	12	16

* T - Toxic.

Table 23

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - PHENOL
EXPERIMENT 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.7	100	6.5	14
	+		4.3	100	2.5	5.9
Pre-treatment Phenol	-	0.0172	5.6	119	8.0	14
	+	0.0172	5.0	116	7.0	14
Post-treatment Phenol	-	0.0118	6.1	130	4.0	6.6
	-	0.0059	4.3	91	5.0	12
	-	0.0030	5.9	126	5.0	8.5
	-	0.0012	6.7	143	5.0	7.5
	+	0.0118	4.4	102	14.0	32
	+	0.0059	4.8	112	5.0	10
	+	0.0030	5.7	133	9.0	16
		0.0012	6.1	142	6.0	9.9

Table 24

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - PHENOL

EXPERIMENT 9

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-5}$)	Per 10^5 Survivors
Negative control	-		7.0	100	2.0	2.9
	+		7.1	100	3.0	4.2
Pre-treatment Phenol	-	0.0172	6.2	89	3.0	4.9
	+	0.0172	6.3	89	5.0	7.9
Post-treatment Phenol	-	0.0055	6.6	94	3.0	4.5
	-	0.0028	5.3	76	2.0	3.8
	-	0.0014	3.9	56	1.0	2.6
	-	0.0006	5.0	71	2.0	4.0
	+	0.0055	5.9	83	6.0	10
	+	0.0028	5.4	76	1.0	1.8
	+	0.0014	4.2	59	3.0	7.1
	+	0.0006	4.5	63	3.0	6.7

Table 25

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - 1,1-DIPHENYLHYDRAZINE
EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		13 8	3 4	13 67	23 57	99 136
Positive controls							
β-Propiolactone	-	10	146				
9-Aminoacridine	-	100		630			
2-Nitrofluorene	-	10			350		
AF2	-	0.1				337	974
2-Anthramine	-	2.5	21	3	15	25	162
	+	2.5	34	32	233	310	490
Pre-treatment	-	169	14	6	7	12	104
1,1-Diphenylhydrazine	+	169	13	4	71	67	99
Post-treatment	-	28	24	2	13	13	137
1,1-Diphenylhydrazine	-	55	16	3	12	21	117
	-	83	22	2	7	23	111
	-	111	15	2	11	31	103
	-	138	17	5	12	25	111
	+	28	4	5	55	27	114
	+	55	14	6	64	17	103
	+	83	9	6	53	20	115
	+	111	5	7	40	42	101
	+	138	16	6	45	38	112

Table 26
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - 1,1-DIPHENYLHYDRAZINE
EXPERIMENT 7

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		33	11	20	31
	+		17	27	27	39
Positive controls						
8-Propiolactone	-	10 µg	74	716		
9-Aminoacridine	-	100			1163	
2-Nitrofluorene	-	50				488
AF2	-	0.1				390
2-Anthramine	+	2.5			200	18
Pre-treatment	-	0.25 ml	25	18	18	127
1,1-Diphenylhydrazine	+	0.25	8	22	53	74
Post-treatment*	-	0.05 ml	28	14	14	23
1,1-Diphenylhydrazine	-	0.10	24	14	12	24
	-	0.15	26	9	23	31
	-	0.20	16	13	14	24
	-	0.25	19	11	17	30
	+	0.05	9	10	49	46
	+	0.10	8	16	45	39
	+	0.15	11	22	40	31
	+	0.20	8	17	24	44
	+	0.25	8	16	47	34

* 100% Reacted.

Table 27

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - 1,1-DIPHENYLHYDRAZINE
EXPERIMENT 8

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	11	14	30
	+		18	9	18	34
Positive controls						
Sodium azide	-	1.0	371	1853		
9-Aminoacridine	-	100				
2-Nitrofluorene	-	50			1162	
AF2	-	0.1				998
2-Anthramine	-	2.5	17	16	13	35
	+	2.5	153	143	1334	1529
Pre-treatment						
1,1-Diphenylhydrazine	-	48.3	19	7	14	14
	+	48.3	16	10	24	34
Post-treatment						
1,1-Diphenylhydrazine	-	5.7	17	12	5	23
	-	11.4	22	16	5	29
	-	17.1	26	20	14	29
	-	22.8	28	33	12	31
	-	28.5	22	36	14	22
	+	5.7	21	7	18	29
	+	11.4	24	10	15	31
	+	17.1	22	14	14	35
	+	22.8	24	32	18	41
	+	28.5	26	35	19	30

Table 23

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - 1,1-DIPHENYLHYDRAZINE
EXPERIMENT 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.7	100	6.5	14
	+		4.3	100	2.5	5.9
Pre-treatment 1,1-Diphenylhydrazine	-	0.0270	8.1	172	5.0	6.2
	+	0.0270	6.8	158	4.0	5.9
Post-treatment 1,1-Diphenylhydrazine	-	0.0221	7.0	149	8.0	11
	-	0.0111	5.4	115	2.0	3.7
	-	0.0055	6.3	134	2.0	3.2
	-	0.0022	5.0	106	2.0	4.0
	+	0.0221	6.1	142	6.0	9.8
	+	0.0111	5.5	128	5.0	9.1
	+	0.0055	6.7	156	9.0	13
	+	0.0022	7.7	179	4.0	5.2

Table 29

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - 1,1-DIPHENYLHYDRAZINE
EXPERIMENT 7

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		2.6	100	5.0	19
	+		2.3	100	2.5	11
Pre-treatment 1,1-Diphenylhydrazine	-	1.0	2.7	104	11.0	38
	+	1.0	2.4	104	8.0	33
Post-treatment* 1,1-Diphenylhydrazine 56	-	1.0	2.1	81	16	76
	-	0.5	3.0	115	6.0	20
	-	0.25	2.6	100	12	46
	-	0.1	2.5	96	8.0	32
	+	1.0	1.8	78	6.0	33
	+	0.5	3.3	143	5.0	15
	+	0.25	2.6	113	9.0	35
	+	0.1	2.7	117	7.0	26

* 100% reacted.

Table 30

IN VITRO ASSAYS WITH SACHAROMYCES CEREVISIAE - 1,1-DIPHENYLHYDRAZINE
EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		6.6	100	3.0	4.5
	+		6.7	100	4.0	6.0
Pre-treatment 1,1-Diphenylhydrazine	-	0.0077	5.9	89	2.0	3.4
	-	0.0077	7.7	115	4.0	5.2
Post-treatment 1,1-Diphenylhydrazine	-	0.0046	6.3	95	1.0	1.6
	-	0.0023	5.8	88	3.0	5.2
	-	0.0011	6.0	91	13.0	22
	-	0.0005	5.1	77	2.0	3.9
	+	0.0046	6.1	91	2.0	3.3
	+	0.0023	5.6	84	4.0	7.1
	+	0.0011	4.8	72	6.0	13
	+	0.0005	5.3	79	4.0	7.5

Table 31
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURUM - BENZOIC ACID
EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		13	3	13	23
	+		8	4	67	57
						136
Positive controls						
β-Propiolactone	-	10	146			
9-Aminoacridine	-	100		630		
2-Nitrofluorene	-	50			350	
AF2	-	0.1				337
2-Anthramine	-	2.5	21	3	15	25
	+	2.5	34	32	233	310
						490
Pre-treatment	-	113	15	3	5	25
Benzoic acid	+	113	7	9	11	31
						102
Post-treatment	-	18	9	6	10	11
Benzoic acid	-	37	24	4	3	16
	-	55	13	7	7	10
	-	73	16	5	8	31
	-	91	8	3	6	23
						86
	+	18	15	5	23	9
	+	37	7	4	12	29
	+	55	11	4	17	25
	+	73	17	8	19	23
	+	91	13	4	23	15
						98

Table 32
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - BENZOIC ACID
EXPERIMENT 8

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	11	14	30
	+		18	9	18	34
Positive controls						
Sodium azide	-	1.0	371	1853	1162	506
9-Aminoacridine	-	100				998
2-Nitrofluorene	-	50				153
AF2	-	0.1				1602
2-Anthramine	-	2.5	17	16	13	35
	+	2.5	153	143	1334	1529
Pre-treatment	-	139.3	17	9	10	23
Benzoic acid	+	139.3	11	8	16	23
Post-treatment	-	25.1	19	6	13	25
Benzoic acid	-	50.2	24	9	11	29
	-	75.4	18	9	6	30
	-	100.5	13	8	12	21
	-	125.6	10	6	9	19
	+	25.1	19	5	12	33
	+	50.2	19	10	21	28
	+	75.4	18	8	12	34
	+	100.5	13	11	17	31
	+	125.6	27	9	17	29

Table 33

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BENZOIC ACID
EXPERIMENT 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.7	100	6.5	14
	+		4.3	100	2.5	5.9
Pre-treatment Benzoic acid	-	0.0180	7.9	168	2.0	2.5
	+	0.0180	5.4	126	6.0	11
Post-treatment Benzoic acid	-	0.0145	7.6	162	7.0	9.2
	-	0.0073	7.6	162	6.0	7.9
	-	0.0037	8.4	179	2.0	2.4
	-	0.0015	6.7	143	2.0	3.0
	+	0.0145	7.4	172	7.0	9.5
	+	0.0073	5.4	126	3.0	5.6
	+	0.0037	6.6	153	3.0	4.5
	+	0.0015	5.8	135	1.0	1.7

Table 34

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BENZOIC ACID
EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.6	100	3.0	4.5
	+		6.7	100	4.0	6.0
Pre-treatment Benzoic acid	-	0.022	6.7	102	5.0	7.5
	+	0.022	7.1	106	3.0	4.2
Post-treatment Benzoic acid	-	0.020	7.6	115	3.0	4.0
	-	0.010	3.9	59	1.0	2.6
	-	0.005	4.8	73	1.0	2.1
	-	0.002	5.8	88	<1.0	1.7
	+	0.020	5.5	82	3.0	5.5
	+	0.010	5.1	76	2.0	3.9
	+	0.005	4.4	66	3.0	6.8
	+	0.002	3.8	58	1.0	2.6

Table 35

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - DIETHYLAMINE
EXPERIMENT 4

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		24	18	8	25 151
	+		15	18	17	34 134
Positive controls						
β-Propiolactone	-	50	451			
9-Aminoacridine	-	100		2625		
2-Nitrofluorene	-	50			1389	
AF2	-	0.1				243 860
2-Anthramine	-	2.5	17	19	16	30 196
	+	2.5	251	202	1529	1620 1730
Pre-treatment Diethylamine	-	143.3	24	23	12	22 139
	+	143.3	20	25	21	23 148
Post-treatment Diethylamine	-	23.8	24	32	13	39 104
	-	47.6	22	21	10	22 103
	-	71.3	13	22	13	22 155
	-	95.1	21	26	10	19 128
	-	118.9	20	18	14	29 144
	+	23.8	10	27	20	18 159
	+	47.6	11	23	17	21 143
	+	71.3	17	14	18	35 132
	+	95.1	13	14	22	45 165
	+	118.9	22	9	12	28 138

Table 36

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - DIETHYLAMINE
EXPERIMENT 14

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		15	5	8	28	114
	+		8	6	17	30	129
Positive controls							
Sodium azide	-	1.0	267	1823			1003
9-Aminoacridine	-	100			1390	249	
2-Nitrofluorene	-	50			380	460	810
2-Anthramine	+	2.5					
Pre-treatment Diethylamine	-	143	14	7	10	26	121
	+	143	6	9	15	24	79
Post-treatment Diethylamine	-	19	9	10	7	20	108
	-	38	15	6	9	22	133
	-	57	15	4	8	27	91
	-	75	14	7	8	14	133
	-	94	11	4	11	11	95
	+	19	9	5	15	22	134
	+	38	8	5	17	26	105
	+	57	7	5	15	18	108
	+	75	5	5	16	20	127
	+	94	6	6	8	29	127

Table 37

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - DIETHYLAMINE
EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		7.4	100	6.0	8.1
	+		8.0	100	5.0	6.3
Pre-treatment Diethylamine	-	0.0229	6.6	89	4.0	6.1
	+	0.0229	6.5	81	5.0	7.7
Post-treatment Diethylamine	-	0.0190	8.9	120	3.0	3.4
	-	0.0095	8.2	111	1.0	1.2
	-	0.0048	9.4	127	3.0	3.2
	-	0.0019	4.7	64	4.0	8.5
	+	0.0190	9.5	119	4.0	4.2
	+	0.0095	8.0	100	3.0	3.8
	+	0.0048	8.0	100	4.0	5.0
	+	0.0019	6.3	79	5.0	7.9

Table 38

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - DIETHYLAMINE
EXPERIMENT 14

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	2.5	4.2
	+		6.5	100	6.5	10
Pre-treatment Diethylamine	-	0.0228	5.5	93	1.0	1.8
	+	0.0228	3.8	58	3.0	7.9
Post-treatment Diethylamine	-	0.0150	0.4	7	2.0	50
	-	0.0075	3.3	56	2.0	6.1
	-	0.0038	5.1	86	1.0	2.0
	-	0.0015	3.5	59	1.0	2.8
	+	0.0150	2.0	31	7.0	35
	+	0.0075	3.5	54	1.0	2.9
	+	0.0038	2.7	42	1.0	3.7
	+	0.0015	4.3	66	2.0	4.7

Table 39
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - HYDROQUINONE
EXPERIMENT 14

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		15	5	8	28 114
	+		8	6	17	30 129
Positive controls						
Sodium azide	-	1.0	267	1823		1003
9-Aminoacridine	-	100			1390	249
2-Nitrofluorene	-	50			380	460
2-Anthramine	+	2.5				810
Pre-treatment Hydroquinone	-	197	T*	8	2	10 T
	+	197	6	4	16	18 122
Post-treatment Hydroquinone	-	20	T	T	T	T T
	-	41	T	T	T	T T
	-	61	T	T	T	T T
	-	82	T	T	T	T T
	-	102	T	T	T	T T
	+	20	4	11	20	28 126
	+	41	7	11	15	25 135
	+	61	9	7	16	24 124
	+	82	T	T	T	T T
	+	102	T	T	T	T T

* T, toxic.

Table 40

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - HYDROQUINONE
EXPERIMENT 17

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate		
			<u>TAL537</u>	<u>TA98</u>	<u>TAL00</u>
Negative control	-		8	25	159
	+		8	38	133
Positive controls					
9-Aminoacridine	-	100	2071		
AF2	-	0.1		46	46
2-Anthramine	-	2.5	11	31	266
	+	2.5	30	1718	1431
Pre-treatment	-	192	10	36	121
Hydroquinone	+	192	6	39	130
Post-treatment	-	16	T*	T	T
Hydroquinone	-	32	T	T	T
	-	48	T	T	T
	-	65	T	T	T
	-	81	T	T	T
	+	16	-	39	124
	+	32	6	33	119
	+	48	9	29	120
	+	65	9	46	139
	+	81	11	47	124

* T, toxic.

Table 41

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - HYDROQUINONE
EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-5}$)	Per 10^5 Survivors
Negative control	-		7.4	100	6.0	8.1
	+		8.0	100	5.0	6.3
Pre-treatment Hydroquinone	-	0.033	4.2	57	6.0	14
	+	0.033	8.2	103	2.0	2.4
Post-treatment Hydroquinone	-	0.026	T*	T	T	T
	-	0.013	T	T	T	T
	-	0.007	3.1	42	8.0	26
	-	0.003	5.1	69	4.0	7.8
	+	0.026	T	T	T	T
	+	0.013	T	T	T	T
	+	0.007	1.9	24	3.0	16
	+	0.003	5.8	73	6.0	10

* T, toxic.

Table 42

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - HYDROQUINONE
EXPERIMENT 14

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	2.5	4.2
	+		6.5	100	6.5	10
Pre-treatment Hydroquinone	-	0.0315	5.9	100	13	22
	+	0.0315	5.7	88	1.7	2.9
Post-treatment Hydroquinone	-	0.0164	T*	T	T	T
	-	0.0082	T	T	T	T
	-	0.0041	3.2	54	9.0	28
	-	0.0016	7.1	120	5.0	7.0
	+	0.0164	T	T	T	T
	+	0.0082	T	T	T	T
	+	0.0041	1.9	29	2.0	11
	+	0.0016	6.7	103	4.0	6.0

* T, toxic.

Table 43

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - HYDROQUINONE

EXPERIMENT 17

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	5.0	9.3
	+		4.2	100	5.0	12
Pre-treatment Hydroquinone	-	0.0308	4.1	76	<1.0	2.4
	+	0.0308	3.8	90	2.0	5.3
Post-treatment Hydroquinone	-	0.0129	T*	T	T	T
	-	0.0065	T	T	T	T
	-	0.0032	T	T	T	T
	-	0.0013	2.1	39	1.0	4.9
	+	0.0129	T	T	T	T
	+	0.0065	T	T	T	T
	+	0.0032	T	T	T	T
	+	0.0013	2.6	62	3.0	12

* T, toxic.

Table 44

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - CAFFEINE
EXPERIMENT 4

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		24	18	8	25 151
	+		15	18	17	34 134
Positive controls						
β-Propiolactone	-	50	451			
9-Aminoacridine	-	100		2625		
2-Nitrofluorene	-	50			1389	
AF2	-	0.1				243 860
2-Anthramine	-	2.5	17	19	16	30 196
	+	2.5	251	202	1529	1620 1730
Pre-treatment	-	111.8	15	21	15	24 105
Caffeine	+	111.8	17	17	18	32 112
Post-treatment	-	22.4	15	18	14	28 121
Caffeine	-	44.7	18	11	4	23 113
	-	67.1	19	22	19	20 130
	-	89.4	20	17	13	20 135
	-	111.8	22	10	10	29 132
	+	22.4	6	22	19	27 103
	+	44.7	20	30	19	30 99
	+	67.1	11	28	16	31 137
	+	89.4	15	12	28	34 130
	+	111.8	11	20	22	31 123

Table 45
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - CAFFEINE
EXPERIMENT 14

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		15	5	8	28
	+		8	6	17	30
						114
						129
Positive controls						
Sodium azide	-	1.0	267	1823		1003
9-Aminoacridine	-	100			1390	249
2-Nitrofluorene	-	50			380	460
2-Anthramine	+	2.5				810
Pre-treatment Caffeine	-	121	26	5	8	16
	+	121	4	2	18	27
						131
						132
Post-treatment Caffeine	-	24	16	4	37	27
	-	48	16	11	31	17
	-	73	14	8	45	49
	-	97	15	4	15	47
	-	121	16	6	14	26
						134
	+	24	11	8	13	19
	+	48	7	7	13	26
	+	73	9	7	19	24
	+	97	5	7	20	25
	+	121	16	4	20	24
						100
						105
						125
						142
						104

Table 46

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - CAFFEINE
EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.4	100	6.0	8.1
	+		8.0	100	5.0	6.3
Pre-treatment Caffeine	-	0.020	7.8	105	6.0	7.7
	+	0.020	7.0	88	4.0	5.7
Post-treatment Caffeine	-	0.020	8.8	119	6.0	6.8
	-	0.010	8.2	111	11	13
	-	0.005	8.5	115	3.0	3.5
	-	0.002	8.1	109	5.0	6.2
	+	0.020	7.5	94	7.0	9.3
	+	0.010	6.9	86	6.0	8.7
	+	0.005	7.6	95	2.5	3.3
	+	0.002	7.2	90	3.0	4.2

Table 47

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - CAFFEINE
EXPERIMENT 14

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	2.5	4.2
	+		6.5	100	6.5	10
Pre-treatment Caffeine	-	0.0194	4.6	78	4.0	8.7
	+	0.0194	4.3	66	3.0	7.0
Post-treatment Caffeine	-	0.0194	T*	T	T	T
	-	0.0097	2.7	46	4.0	15
	-	0.0048	4.6	78	3.0	6.5
	-	0.0019	4.1	69	1.0	2.4
	+	0.0194	T	T	T	T
	+	0.0097	2.6	40	1.0	3.8
	+	0.0048	4.1	63	1.0	2.4
	+	0.0019	4.3	66	4.0	9.2

74

* T, toxic.

Table 48
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - BENZIDINE
EXPERIMENT 6

Compound	Metabolic Activation	Amount of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA98 TA100
Negative control	-		17	11	16	25 133
	+		11	16	17	37 134
Positive controls						
β-Propiolactone	-	10 μg	802			
9-Aminoacridine	-	100		1122		
2-Nitrofluorene	-	50			1853	
AF2	-	0.1				250 1111
2-Anthramine	-	2.5	18	9	13	34 154
	+	2.5	60	24	266	285 609
Pre-treatment	-	0.25 ml	12	10	6	13 136
Benzidine	+	0.25	15	5	60	62 159
Post-treatment*	-	0.05 ml	15	7	11	2 181
Benzidine	-	0.10	T†	T	T	T T
	-	0.15	T	T	T	T T
	-	0.20	T	T	T	T T
	-	0.25	T	T	T	T T
	+	0.05	10	11	14	29 130
	+	0.10	17	6	9	22 145
	+	0.15	T	6	8	21 128
	+	0.20	T	4	12 T	21 T 94
	+	0.25	6 T	T	45 T	1 T 93

* 100% Reacted.
† T, toxic.

Table 49

IN VITRO ASSAYS WITH *SALMONELLA* TYPHIMURIUM - BENZIDINE
EXPERIMENT 12

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	5	8	112
	+		10	6	9	85
Positive controls						
Sodium azide	-	1.0	360			
9-Aminoacridine	-	100		1675		
2-Nitrofluorene	-	50			654	694
AF2	-	0.1	19	5	12	145
2-Anthramine	-	2.5	149	153	806	1704
	+	2.5				
Pre-treatment Benzidine	-	46.5	19	8	8	94
	+	46.5	11	8	15	97
Post-treatment Benzidine	-	3.8	24	11	5	149
	-	7.6	19	1	6	134
	-	11.4	11	7	14	160
	-	15.3	4	4	5	198
	-	19.1	8	4	5	143
	+	3.8	8	5	12	122
	+	7.6	12	1	10	99
	+	11.4	15	4	16	107
	+	15.3	10	4	9	138
	+	19.1	11	2	11	112

Table 50

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BENZIDINE
EXPERIMENT 12

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.2	100	1.0	1.9
	+		5.7	100	3.0	5.3
Pre-treatment Benzidine	-	0.00074	7.5	144	1.0	1.3
	+	0.00074	6.4	112	2.0	3.1
Post-treatment Benzidine	-	0.0031	6.2	119	2.0	3.2
	-	0.0015	7.1	137	< 1.0	1.4
	-	0.0008	6.0	115	3.0	5.0
	-	0.0003	8.0	154	2.0	2.5
	+	0.0031	7.2	126	1.0	1.4
	+	0.0015	6.6	116	1.0	1.5
	+	0.0008	7.8	137	1.0	1.3
	+	0.0003	6.0	105	2.0	3.3

Table 51

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BENZIDINE

EXPERIMENT 16

Compound	Metabolic Activation	Milliliters of Solution Added	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		3.5	100	5.0	14
	+		3.2	100	6.0	19
Pre-treatment Benzidine	-	1.0	3.7	106	3.0	8.1
	+	1.0	3.6	113	6.0	17
Post-treatment* Benzidine	-	1.0	T†	T	T	T
	-	0.5	T	T	T	T
	-	0.25	T	T	T	T
	-	0.1	0.7	2	< 1.0	14
	+	1.0	T	T	T	T
	+	0.5	T	T	T	T
	+	0.25	T	T	T	T
	+	0.1	2.3	72	4.0	17

* 100% reacted.

† T, toxic.

Table 52
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - UREA
EXPERIMENT 4

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		24	18	8	25	151
	+		15	18	17	34	134
Positive controls							
8-Propiolactone	-	50	451				
9-Aminoacridine	-	100		2625			
2-Nitrofluorene	-	50			1389	243	860
AF2	-	0.1				30	196
2-Anthramine	-	2.5	17	19	16	1620	1730
	+	2.5	251	202	1529		
Pre-treatment	-	143.5	11	10	17	17	151
Urea	+	143.5	13	27	17	25	170
Post-treatment	-	28.7	23	29	21	36	102
Urea	-	57.4	25	15	15	28	126
	-	86.1	21	18	9	20	104
	-	114.8	22	21	13	23	141
	-	143.5	18	27	19	27	102
	+	28.7	12	17	16	35	135
	+	57.4	12	11	22	30	136
	+	86.1	15	24	27	34	104
	+	114.8	11	26	22	32	122
	+	143.5	10	19	20	46	115

Table 53
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - UREA
EXPERIMENT 14

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		15	5	8	114
	+		8	6	17	129
Positive controls						
Sodium azide	-	1.0	267	1823		1003
9-Aminoacridine	-	100			1390	
2-Nitrofluorene	-	50			380	
2-Anthramine	+	2.5			249	810
Pre-treatment Urea	-	144.5	14	6	3	105
	+	144.5	14	2	14	106
Post-treatment Urea	-	28.9	23	3	15	127
	-	57.8	20	6	8	119
	-	86.7	18	8	5	150
	-	115.6	17	8	6	139
	-	144.5	22	7	6	146
	+	28.9	13	7	17	127
	+	57.8	13	6	12	92
	+	86.7	5	4	16	124
	+	115.6	7	7	13	100
	+	144.5	10	3	16	131

Table 54
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - UREA
EXPERIMENT 18

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		25	13	8	133
	+		14	6	16	98
Positive controls						
Sodium azide	-	1.0	694			
9-Aminoacridine	-	100		371		
2-Nitrofluorene	-	50			977	
AF2	-	0.1				1223
2-Anthramine	-	2.5		6	8	94
	+	2.5		82	912	1079
Pre-treatment	-					
Urea	+	136.5	17	12	12	122
		136.5	8	6	16	C*
Post-treatment	-					
Urea	-	27.3	28	9	9	133
	-	54.6	23	11	13	116
	-	81.9	38	15	7	106
	-	109.2	20	20	7	100
	-	136.5	28	14	7	86
	+	27.3	12	14	19	114
	+	54.6	7	12	21	117
	+	81.9	9	11	24	107
	+	109.2	8	11	27	104
	+	136.5	10	8	16	116

* C, contaminated.

Table 55

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - UREA
EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^3 Survivors
Negative control	-		7.4	100	6.0	8.1
	+		8.0	100	5.0	6.3
Pre-treatment Urea	-	0.023	7.8	105	3.0	3.9
	+	0.023	8.4	105	3.0	3.6
Post-treatment Urea 82	-	0.023	7.1	96	4.0	5.6
	-	0.011	7.0	95	2.0	2.9
	-	0.006	7.8	105	3.0	3.9
	-	0.002	7.6	103	8.0	11
	+	0.023	7.1	89	<1.0	1.4
	+	0.011	5.9	74	3.0	5.1
	+	0.006	6.8	85	4.0	5.9
	+	0.002	6.9	86	4.0	5.8

Table 56

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - UREA

EXPERIMENT 14

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	2.5	4.2
	+		6.5	100	6.5	10
Pre-treatment Urea	-	0.023	4.3	73	1.0	2.3
	+	0.023	3.9	60	2.0	5.1
Post-treatment Urea	-	0.023	2.4	41	1.0	4.2
	-	0.012	4.0	68	1.0	2.5
	-	0.006	4.0	68	1.0	2.5
	-	0.002	3.7	63	3.0	8.1
	+	0.023	3.3	51	1.0	3.0
	+	0.012	3.4	52	3.0	8.8
	+	0.006	3.3	51	3.0	9.1
	+	0.002	3.2	49	1.0	3.1

Table 57

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - UREA
EXPERIMENT 18

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		4.7	100	5.0	11
	+		5.5	100	4.5	8.2
Pre-treatment Urea	-	0.0218	5.6	119	5.0	8.9
	+	0.0218	5.0	91	1.0	2.0
Post-treatment Urea	-	0.0218	4.4	94	9.0	21
	-	0.0109	4.6	98	1.0	2.2
	-	0.0055	3.9	83	5.0	13
	-	0.0022	3.9	83	3.0	7.7
	+	0.0218	4.0	73	10.0	25
	+	0.0109	4.7	85	4.0	8.6
	+	0.0055	4.7	85	1.0	2.1
	+	0.0022	4.3	78	5.0	12

Table 58

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - BENZENE*
EXPERIMENT 6

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			TA1535	TA1537	TA1538	TA100
Negative control	-		17	11	16	25
	+		11	16	17	37
Positive controls						
β-Propiolactone	-	10	802	1122	1853	250
9-Aminoacridine	-	100				1111
2-Nitrofluorene	-	50				154
AF2	-	0.1	18	9	13	34
2-Anthramine	-	2.5	60	24	266	285
	+	2.5				
Post-treatment Benzene	-	6.5	20	16	13	25
	-	13.0	16	12	10	19
	-	19.5	23	11	13	23
	-	26.0	29	8	20	30
	-	32.5	14	6	7	12
	+	6.5	9	11	15	28
	+	13.0	11	13	20	43
	+	19.5	12	9	10	33
	+	26.0	9	14	13	32
	+	32.5	10	8	15	28

* Pre-treatment experiment contaminated.

Table 59

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - BENZENE
EXPERIMENT 17

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		24	8	13	25	159
	+		13	8	23	38	133
Positive controls							
Sodium azide	-	1.0	912	2071	1520		46
9-Aminoacridine	-	100					266
2-Nitrofluorene	-	50					1431
AF2	-	0.1	17	11	16	46	
2-Anthramine	-	2.5	391	30	454	1718	
	+	2.5					
Pre-treatment Benzene	-	55.3	25	8	8	29	152
	+	55.3	13	3	8	51	135
Post-treatment Benzene	-	8.7	18	11	11	31	122
	-	17.5	26	17	9	24	154
	-	26.2	27	7	12	25	126
	-	34.9	18	11	10	31	154
	-	43.6	22	9	13	20	136
	+	8.7	7	9	18	40	131
	+	17.5	14	9	14	40	130
	+	26.2	7	7	17	41	135
	+	34.9	14	5	11	31	119
	+	43.6	15	6	14	46	135

Table 60

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BENZENE
EXPERIMENT 6

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		3.5	100	5.0	14
	+		3.2	100	6.0	19
Pre-treatment Benzene	-	0.0140	3.8	109	4.0	11
	+	0.0140	2.9	91	5.0	17
Post-treatment Benzene	-	0.0052	3.3	94	5.0	15
	-	0.0026	3.6	103	6.0	17
	-	0.0013	3.6	103	6.0	17
	-	0.0005	3.9	111	3.0	7.7
	+	0.0052	3.0	94	2.0	6.7
	+	0.0026	3.3	103	4.0	12
	+	0.0013	3.4	106	7.0	21
	+	0.0005	3.3	103	5.0	15

Table 61

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - BENZENE
EXPERIMENT 10

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.4	100	1.0	1.9
	+		3.5	100	1.0	2.9
Pre-treatment Benzene	-	0.0191	5.4	100	1.0	1.9
	+	0.0191	5.0	93	1.0	2.0
Post-treatment Benzene	-	0.0088	4.8	89	<1.0	2.1
	-	0.0044	4.5	83	1.0	2.2
	-	0.0022	5.5	102	<1.0	1.8
	-	0.0009	5.1	94	<1.0	2.0
	+	0.0088	4.6	135	4.0	8.7
	+	0.0044	3.4	97	1.0	2.9
	+	0.0022	6.5	186	1.0	1.5
	+	0.0009	4.4	126	3.0	6.8

Table 62

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - OLEIC ACID
EXPERIMENT 5

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		16	13	16	37	136
	+		12	20	15	47	135
Positive controls							
β-Propiolactone	-	10	61				
9-Aminoacridine	-	100		1299			
2-Nitrofluorene	-	50			753		
AF2	-	0.1				45	197
2-Anthramine	-	2.5	9		15	41	115
	+	2.5	223		1546	1385	1877
Pre-treatment Oleic acid	-	5.3	15	8	5	22	116
	+	5.3	6	8	11	37	107
Post-treatment Oleic acid	-	0.13	14	16	14	26	142
	-	0.26	7	18	10	41	126
	-	0.39	16	13	17	23	105
	-	0.52	10	18	14	34	118
	-	0.65	7	5	16	21	81
	+	0.13	15	9	13	35	106
	+	0.26	13	16	14	34	121
	+	0.39	13	17	6	33	147
	+	0.52	14	17	12	44	179
	+	0.65	11	4	13	37	162

Table 63.

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM - OLEIC ACID
EXPERIMENT 18

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		25	13	8	13	133
	+		14	6	16	31	98
Positive controls							
Sodium azide	-	1.0					
9-Aminoacridine	-	100					
2-Nitrofluorene	-	50		371			
AF2	-	0.1			977		
2-Anthramine	-	2.5	25	6	8	495	1223
	+	2.5	18	82	912	20	94
						945	1079
Pre-treatment	-	5.5	25	14	10	23	143
Oleic acid	+	5.5	11	5	19	24	110
Post-treatment	-	0.33	28	11	11	14	144
Oleic acid	-	0.66	25	24	6	18	153
	-	0.99	22	12	8	22	134
	-	1.32	21	20	14	19	154
	-	1.65	31	9	12	19	110
	+	0.33	9	9	20	28	120
	+	0.66	9	12	19	46	131
	+	0.99	8	11	20	46	101
	+	1.32	8	10	26	36	130
	q	1.65	11	8	24	57	107

Table 64

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE - OLEIC ACID

EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.0	100	6.5	13
	+		6.2	100.	6.5	10
Pre-treatment Oleic acid	-	0.00084	6.5	130	2.0	3.1
	+	0.00084	6.5	105	6.7	10
Post-treatment Oleic acid	-	0.00010	5.4	108	4.0	7.4
	-	0.00005	7.0	140	4.0	5.7
	-	0.00003	5.9	118	6.0	10
	-	0.00001	5.8	116	3.8	6.6
	+	0.00010	5.2	84	5.0	9.6
	+	0.00005	4.9	79	4.0	8.2
	+	0.00003	5.2	84	10.0	19
	+	0.00001	4.2	68	6.0	14